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MAXIMUM RATE ANALYSIS OF ERYTHROCYTE
CARBONIC ANHYDRASE IN HYPERCAPNIC GUINEA PIGS

by

Donald V. Tappan

Released by:

Gerald J. Duffner, CAPT MC USN
COMMANDING OFFICER
Naval Submarine Medical Center

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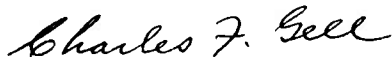
by

Donald V. Tappan, Ph.D.

SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 568

Submitted by:

Karl E. Schaefer, M.D.

Reviewed and Approved by:

Charles F. Gell

Charles F. Gell, M.D., D.Sc.
Scientific Director, SubMedResLab

Reviewed and Approved by:

Joseph D Bloom

J. D. Bloom, CDR MC USN
Director, SubMedResLab

Approved and Released by:

Gerald J. Duffner

Gerald J. Duffner
Captain, Medical Corps, U.S. Navy
COMMANDING OFFICER, SMC

SUMMARY PAGE

THE PROBLEM

To evaluate enzymic adaptations to stresses, specifically adaptation of the carbonic anhydrases to high carbon dioxide environments, occurring in the absence of gross changes in measurable enzymic activity.

FINDINGS

Adaptive mechanisms are operative for the maintenance of normal levels of carbonic anhydrase activity in the erythrocytes of guinea pigs exposed for up to seven days to atmospheres containing 15% CO_2. Such processes tend to reduce the activity per mole of enzyme when enzyme levels are increased as a result of increases in erythrocyte concentration during early periods of exposure and to activate remaining enzyme molecules as the supply diminishes through use or lack of replacement.

APPLICATIONS

The new type of adaptive mechanism described increases the possibility of making useful predictions concerning the ability of men to work under conditions of severe stress.

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ABSTRACT

Studies have been made of the activity and concentration of erythrocyte carbonic anhydrase in guinea pigs exposed for up to seven days to atmospheres containing 15% CO_2. Data are presented which indicate that adaptive processes tend to reduce the activity per mole of enzyme when the enzyme concentration is increased by such processes as hemoconcentration, i.e., during the first 24-48 hours of CO_2 exposure, and to increase activity per mole when enzyme concentration is reduced through use or degenerative processes, or after about seven days of exposure.

Several general interpretations are proposed for utilization of data from analyses of a non-linear activity vs enzyme concentration relationship.

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INTRODUCTION

Since enzyme systems are often modified in their activity or protein content by the level of substrate available upon which to operate,[1] the effect of high carbon dioxide environments upon the carbonic anhydrases is a subject of considerable theoretical and practical importance. Although many guinea pigs are able to live for seven days or longer when exposed to atmospheres containing 15% CO_2, others cannot adapt well enough to remain alive under such conditions. This may be taken, then, as one of the most drastic situations with regard to environmental carbon dioxide which this species can endure. If carbonic anhydrase is able to play any useful role toward survival under very rigorous conditions, the surviving animals should provide evidence of enzymic adaptation.

Previous attempts to demonstrate differences in the activity of carbonic anhydrase or in its forms or content in the erythrocytes or other tissues of guinea pigs living in high CO_2 environments have generally produced negative or inconclusive results because of the wide variability between individual animals which has regularly proved to be greater than differences between variously treated groups.[2,3] To overcome part of the difficulties of interpretation of isolated measurements, analyses in the present study were made over a broad range of enzyme concentrations, even beyond the range providing linear proportionality between activity and enzyme level. Calculation of the results has been possible by applying the theoretical considerations provided by Darvey, Prokhovnik and Williams[4] whose "maximum rate" kinetic analysis allows for a non-linear relationship between enzyme concentration and activity, i.e., the effect on activity of wide ranges of enzyme concentration.

METHODS AND MATERIALS

Carbonic anhydrase activity analyses have been made by an adaptation of the principle originally utilized by Philpot and Philpot[5] in which carbon dioxide is fed into a buffered reaction mixture as a regulated stream in order to maintain a constant level of substrate. A simple reaction mixture consisting of 0.15 M tris (hydroxymethyl) aminomethane-acetate in the presence of the diluted tissue preparations was employed with a glass electrode and a recording system for determining the time required for the pH of the medium to be changed at 30° from 7.68 to 7.48.

For the activity determinations, whole blood was hemolyzed by 1:50 dilution with distilled-deionized water and routinely analyzed at 12 levels over a 60-fold concentration range. The method of Straus and Goldstein[6] and Maren[7] was utilized with ethoxyzolamide* as the inhibitor at ten dilutions over the range of 4 x 10^{-9} to 5.5 x 10^{-8} against 100 ul of the diluted hemolysate per 1.5 ml reaction mixture for the measurement of enzyme concentrations.

Analyses of experimental data were based on a reciprocal form of the enzyme-dependent kinetic equation of Darvey, et al.[4], in

$$\frac{E}{v} = \frac{K_e}{V_e} + \frac{E}{V_e} \qquad (1),$$

which K_e and V_e represent constants defining the relationship between velocity, v, and enzyme concentration, E.

RESULTS

The data for both reaction constants and erythrocyte enzyme content (at the diluted level) for the various groups of animals are summarized in Table I. Demonstrable differences exist between the enzyme levels in the two groups of exposed animals with an apparent difference between the K_e values for the control and the 7-day exposed groups. A difference that approaches significance between the V_e/K_e data of the control and the 1-day experimental groups ($p < .10$) has been determined, although not listed sepa-

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*Supplied by the Upjohn Co., Kalamazoo, Mich.

TABLE I. MAXIMUM RATE KINETIC DATA AND ENZYME CONCENTRATION
FOR CO$_2$-EXPOSED AND CONTROL GUINEA PIGS

	Group I Control	Group II 1-day Exp.	Group III 7-day Exp.	Significance Test Groups	p of t
Number of Animals	16	13	11		
$K_e \times 10^8$, M	$3.254 \pm .460$	$3.939 \pm .450$	$2.584 \pm .395$	I— II I—III II—III	n.s.* <.05 n.s.
$V_e \times 10^3$, moles $1^{-1} sec^{-1}$	$7.066 \pm .397$	$6.653 \pm .315$	$5.870 \pm .804$	I— II I—III II—III	n.s. n.s. n.s.
$E \times 10^8$, M	$1.925 \pm .176$	$2.210 \pm .110$	$1.536 \pm .212$	I— II I—III II—III	n.s. n.s. <.01
Activity at in vitro conc., moles $1^{-1} sec^{-1}$	$2.673 \pm .154$	$2.458 \pm .128$	$2.036 \pm .129$	I— II I—III II—III	n.s. <.01 <.05
Activity at 1×10^{-8}M, moles $1^{-1} sec^{-1}$	$1.914 \pm .226$	$1.445 \pm .116$	$1.987 \pm .482$	I— II I—III II—III	<.10>.05 n.s. n.s.

*n.s. = Nul probability greater than ten percent

Note: Data are presented ± std. error of mean (8). Calculations were made from
Eqn 1 by the modified least squares analysis of Smith and Mathews (9).

rately in Table I. In order to more fully evaluate the data, reaction velocities for the enzyme system in the various groups of animals were calculated employing the experimentally derived constants, V_e and K_e, in Eqn 1 which may be rewritten:

$$v = \frac{V_e E}{K_e + E} \qquad (2).$$

Activity values are given in Table I for the enzyme concentrations near the midpoint of the ranges studied, i.e., at the levels at which enzyme concentrations were determined, and at a constant enzyme level, 1×10^{-8} M.

DISCUSSION AND CONCLUSIONS

While enzyme activity differences exist between the control and the 7-day and between the 1- and 7-day exposed groups of animals for the in vitro enzyme concentrations shown in the Table, no conclusions may be drawn concerning the significance of such differences at the enzyme concentrations within the intact erythrocytes. In fact, the large discrepancy between potential carbonic anhydrase activity and the activity utilized during the normal circulation of the erythrocytes (10-11) would tend to minimize the influence of differences in enzyme concentration at the high levels occurring in the erythrocytes. It is worthy of note that calculations made according to Eqn 2 at enzyme

concentrations approaching those of erythrocytes show no meaningful differences in activity at such levels.

Despite the problems still unresolved concerning any adaptation of guinea pigs to the carbon dioxide-rich environment through changes in enzyme activity, considerable information may be gleaned from the data pertaining to the methods operating within this biological system for utilizing an apparently generous reserve of catalytic activity without dependence upon production of new protein for adaption.

While such simplifications are limited in value, in a pragmatic sense, K_e may be considered to be related to the affinity of enzyme and substrate in the enzyme-substrate complex (cf. interpretations that have been made for the constants, V_m and K_m, ref. 1). The probable reduction in K_e for the 7-day exposed animals would support the concept that some change has occurred in the nature of the enzyme, perhaps amounting to a slight denaturation, during the seven days of overstimulation or to a general deterioration resulting from the stress. Very slight changes in the structure of the proteins are proposed since no differences in electrophoretic mobility of the enzymes have been observed under these conditions.

The apparent increase in enzyme content per volume of blood for the 1-day exposed guinea pigs may be taken as a direct reflection of the hemoconcentration that occurs in these animals during the first 24 hours of CO_2 exposure[12]. On the other hand, the lowered level of enzyme at seven days seems to support the concept of a denaturation of some of the normal enzyme protein or a failure to maintain mechanisms required for normal repair and replacement during the exposure period.

An interesting interpretation of the K_e and V_e changes may be made from consideration of the V_e/K_e ratios. The expression describing the period of an enzyme reaction when velocity is proportional to enzyme concentration,

$$v = kE \qquad (3),$$

may be obtained from Eqn 2 when E is small compared to K_e with the ratio of the constants, V_e/K_e, in Eqn 2 equivalent to the single constant, k, in Eqn 3. Besides being valid at very low concentrations of enzyme, Eqn 3 is usually considered to hold during the very early stages of enzyme reactions. While the data presented are only suggestive of an inverse relationship of this ratio to enzyme concentration, in many data for individual or small groups of animals, a strong relationship seemed obvious. In all cases when unusually low values of E were obtained, the V_e/K_e ratios were correspondingly increased. The mean V_e/K_e for 12 animals was 5.11 x 10^5 sec^{-1} when E was less than 1.5 x 10^{-8} M and for the four cases with E less than 1.0 x 10^{-8}, V_e/K_e = 7.84 x 10^5. These data contrast sharply with the mean values of 1.7 — 2.3 x 10^5 sec^{-1} that may be calculated from the information reported in the Table. The reciprocal relationship of activity ratio to enzyme concentration seems to be valid regardless of experimental treatment of the animals and tends to be of greater significance since the reaction constants and enzyme concentrations were determined in separate analyses eliminating much of the possibility of common experimental errors. The phenomenon just described appears to be strong evidence of compensatory changes which tend to maintain the activity of the enzymes at or near a constant level.

It should be mentioned that the V_e/K_e values obtained here fall into the general range of the V_m/E_o data reported by Gibbons and Edsall[13] and considered by Maren[10] to belong to a standard range for maximum activity of high activity carbonic anhydrases, 2 — 6 x 10^5 sec^{-1}.

The last line of the Table strengthens earlier conclusions concerning adjustments of enzyme activity. The lowered level of activity after one day of exposure when compared at a constant level, 1 x 10^{-8} M, suggests that the enzyme does indeed compensate for concentration influences since at this time enzyme concentration itself is maximal.

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At seven days when enzyme concentration is reduced, a small increase in activity per mole of enzyme may have occurred although this has not been shown to be significantly different from the other mean values because of the aforementioned variability among the individual animals after the long exposure period.

One further insight into the adaptation process, implied but not proven by the data, may be gained from the V_e/K_e data. If these values represent maximal initial rates per mole of enzyme, then it may be inferred from the increased ratio at seven days that an adjustment has been initiated in some or all of the enzyme molecules in an attempt to compensate for the lowered enzyme concentration resulting from the prolonged exposure to CO_2. Whatever compensatory mechanisms are involved here would seem to be oppositional to the processes which lead to reduced activity as a result of the increased concentration of the enzymes after the first day of exposure to the stressful environment.

That enzymes or other proteins may have an inherent ability to perform some rudimentary adaptive processes not necessarily dependent upon the synthesis of new protein molecules would seem to be an hypothesis worthy of further consideration.

SUMMARY

Studies have been made of the activity and concentration of erythrocyte carbonic anhydrase in guinea pigs exposed for up to seven days to atmospheres containing 15% CO_2. Data are presented which indicate that adaptive processes tend to reduce the activity per mole of enzyme when the enzyme concentration is increased by such processes as hemoconcentration, i.e., during the first 24 to 48 hours of CO_2 exposure, and to increase activity per mole when enzyme concentration is reduced through use or degenerative processes, or after about seven days of exposure.

Several general interpretations are proposed for utilization of data from analyses of a non-linear activity vs enzyme concentration relationship[2].

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13. ABSTRACT

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